ORIGINAL ARTICLE

Intraventricular administration of urokinase as a novel therapeutic approach for communicating hydrocephalus

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Fibrosis of the subarachnoid space (SAS) after infection, inflammation, or hemorrhage can impair cerebrospinal fluid absorption and circulation, causing diffuse ventricular dilatation. In the present study, we tested the hypothesis that urokinase (also known as urokinase-type plasminogen activator (uPA)), a fibrinolytic agent, attenuates fibrosis and ventriculomegaly in a rat model of kaolin-induced communicating hydrocephalus and thus may have potential as a therapy for these conditions. Thirty microliters of sterile 25% kaolin suspension was injected into the basal cisterns of adult Sprague–Dawley rats to induce hydrocephalus, and 2 intraventricular injections of either uPA or vehicle (saline) were administered immediately and 3 days thereafter. Ventricular volumes were measured by magnetic resonance imaging (MRI) on days 3, 14, and 28 after kaolin injection. Fibrosis and reactive astrogliosis were evaluated on day 28 by immunofluorescence and Western blotting. Neurocognitive features were tested using the Morris water maze from days 23 to 28. MRI analysis demonstrated that kaolin administration successfully induced hydrocephalus in rats and that uPA treatment significantly attenuated ventricular enlargement. In addition, uPA inhibited the deposition of laminin and fibronectin, extracellular matrix molecules, in the SAS, attenuated gliosis, and improved learning and memory in kaolin-treated rats. Therefore, we concluded that uPA prevents the development of kaolin-induced communicating hydrocephalus by preventing the development of subarachnoid fibrosis and by eliciting improvements in neurocognition. The results of this study indicate that uPA may be a novel clinical therapy for communicating hydrocephalus. (Translational Research 2016;■:1–14)

Abbreviations: BVs = blood vessels; CSF = cerebrospinal fluid; ECM = extracellular matrix; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GFAP = glial fibrillary acidic protein; IF = immunofluorescence; LV = lateral ventricle; MRI = magnetic resonance imaging; PBS = phosphate-buffered solution; PL = plasmin; PLG = plasminogen; SAS = subarachnoid space; tPA = tissue-type plasminogen activator; uPA = urokinase-type plasminogen activator; uPAR = urokinase-type plasminogen activator receptor; 3V = third ventricle; WB = Western blot

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AT A GLANCE COMMENTARY

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Background

Communicating hydrocephalus is one of the most common complications after intracranial hemorrhage, intraventricular hemorrhage, and subarachnoid hemorrhage. But there is no efficient treatment for this severe neurologic condition. Previous researches have recognized that fibrosis in the subarachnoid space (SAS) is a crucial pathogenesis of communicating hydrocephalus.

Translational Significance

Urokinase-type plasminogen activator (uPA) is an important component of the fibrinolytic system. It can degrade the extracellular matrix (ECM) components directly and by activating plasminogen to plasmin. Science 1999, although, uPA is not available in the United States, our results of present study show that uPA reduced the deposition of ECM and fibrosis in the SAS, thus preventing the development of communicating hydrocephalus and improving long-term neurocognitive defects. This suggests uPA may be an efficient therapeutic treatment for the prevention of communicating hydrocephalus.

INTRODUCTION

Hydrocephalus is a common neurologic condition that develops secondary to meningitis,[1] germinal matrix hemorrhage,[2] intraventricular hemorrhage,[3] and subarachnoid hemorrhage.[4,5] It is usually caused by impaired cerebrospinal fluid (CSF) flow or drainage and is characterized by pathologic dilation of the cerebral ventricles.[6] Currently, the only definitive therapy for hydrocephalus is CSF shunting. However, shunting is not an ideal treatment because its complications, such as obstruction and infection, result in high revision rates[7] and high frequencies of residual neurologic deficits.[8] Therefore, it is important to develop efficient therapies for hydrocephalus.

Previous studies have determined that extensive fibrosis in the subarachnoid space (SAS), which is characterized by excessive extracellular matrix (ECM) production, is closely associated with posthemorrhagic hydrocephalus and other forms of communicating hydrocephalus.[9,10] Thus, targeting subarachnoid fibrosis may be a good approach to treating communicating hydrocephalus.[11,12]

Urokinase-type plasminogen activator (uPA) is a serine protease that converts plasminogen (PLG) to plasmin (PL). PL is an active protease that can degrade fibrin and ECM components.[13] Activation of the uPA/PL system reportedly exerts beneficial effects in experimental models of lung,[14,15] airway,[16] and liver fibrosis.[17,18] Intraventricular fibrinolysis has also been shown to improve outcomes after intraventricular hemorrhage.[19] For unknown reasons, uPA has not been available in the United States since 1999. However, in recent years, uPA has gradually resurfaced and has received much consideration regarding its usefulness as a fibrinolytic therapeutic agent. uPA-mediated fibrinolysis significantly accelerates hematoma resolution and improves outcomes in animal models of subarachnoid hemorrhage,[20] intraventricular hemorrhage,[21] and intracerebral hemorrhage.[22] However, to date, the effects of uPA on chronic communicating hydrocephalus are unknown.

Therefore, a rat model of kaolin-induced communicating hydrocephalus was used to test the hypothesis that intraventricular uPA infusions can alleviate communicating hydrocephalus by attenuating subarachnoid fibrosis, thereby preventing the brain pathology induced by hydrocephalus and improving long-term neurocognition outcomes.

MATERIALS AND METHODS

Animal preparation and groups. Seventy-two male Sprague–Dawley rats (250–300 g; the Third Military Medical University) were used in the present study. All rats were maintained under standard conditions (23°C–25°C, 70% humidity, 12 hour light–dark cycles) with adequate access to food and water. Animal use protocols were approved by the Animal Care and Use Committee of the Third Military Medical University.

Our experiment was divided into 2 parts. The first part was undertaken to choose the right dose of uPA. Twenty-four rats were randomly assigned to the following 4 groups (n = 6 per group) after kaolin injection: kaolin injection with intraventricular injection of 0.9% sterile saline (kaolin + vehicle group) and kaolin injection with intraventricular injection of 5 IU (low-dose group), 10 IU (middle-dose group), and 20 IU (high-dose group) of uPA. Then, all rats underwent magnetic resonance imaging (MRI) examinations on day 28 after kaolin injection. The procedures for the second part of the experiment are shown in Fig 1, *A*. Rats were randomly assigned to the following 2 groups: a saline injection (sham) group and a kaolin injection group. After kaolin injection, these rats were randomly assigned to kaolin injection only (kaolin group), kaolin injection with intraventricular injection of 0.9% sterile